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Year: 2014

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## **Treating progressive multifocal leukoencephalopathy with interleukin 7 and vaccination with JC virus capsid protein VP1**

Sospedra, Mireia ; Schippling, Sven ; Yousef, Sara ; Jelcic, Ilijas ; Bofill-Mas, Silvia ; Planas, Raquel ; Stellmann, Jan-Patrick ; Demina, Viktoria ; Cinque, Paola ; Garcea, Robert ; Croughs, Therese ; Girones, Rosina ; Martin, Roland

**Abstract:** Progressive multifocal leukoencephalopathy is a currently untreatable infection of the brain. Here, we demonstrate in 2 patients that treatment with interleukin 7, JC polyomavirus (JCV) capsid protein VP1, and a Toll-like receptor 7 agonist used as adjuvant, was well tolerated, and showed a very favorable safety profile and unexpected efficacy that warrant further investigation.

DOI: <https://doi.org/10.1093/cid/ciu682>

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ZORA URL: <https://doi.org/10.5167/uzh-99619>

Journal Article

Accepted Version

Originally published at:

Sospedra, Mireia; Schippling, Sven; Yousef, Sara; Jelcic, Ilijas; Bofill-Mas, Silvia; Planas, Raquel; Stellmann, Jan-Patrick; Demina, Viktoria; Cinque, Paola; Garcea, Robert; Croughs, Therese; Girones, Rosina; Martin, Roland (2014). Treating progressive multifocal leukoencephalopathy with interleukin 7 and vaccination with JC virus capsid protein VP1. *Clinical Infectious Diseases*, 59(11):1588-1592.

DOI: <https://doi.org/10.1093/cid/ciu682>

**Brief Report**

**Treating PML with Interleukin-7 and Vaccination with JC Virus Capsid Protein VP1**

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**Key words:** Progressive Multifocal Leukoencephalopathy, JC virus, IL-7, VP1

## **ABSTRACT**

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Progressive multifocal leukoencephalopathy is a currently untreatable infection of the brain. Here, we demonstrate in two patients that treatment with interleukin-7, JCV capsid protein VP1 and a toll-like receptor 7 agonist used as adjuvant, was well tolerated, showed a very favorable safety profile and unexpected efficacy that warrants further investigation.

## **INTRODUCTION**

Progressive multifocal leukoencephalopathy (PML) is a life-threatening opportunistic infection of the brain caused by JC polyoma virus (JCV) [1] that occurs in various states of immunosuppression. The occurrence of PML in conditions of decreased CD4<sup>+</sup> T cell numbers or function with resolution following their restoration [2] and immunological studies from brain-infiltrating T cells [3] strongly support the importance of these immune cells in controlling JCV infection. JCV-specific CD8<sup>+</sup> cytotoxic T cells also play an important role in controlling this infection and have been linked to recovery from PML [4]. The role of antibodies is less clear, since they are frequently present before and at onset of PML [5]. Currently, there is no specific antiviral- or other drug to treat PML, and the only option is to restore protective immunity. The hematopoietic growth factor interleukin-7 (IL-7) is crucial for homeostatic T cell proliferation [6] and restores T cell function including virus-specific immunity [7]. Since vaccines induce antigen-specific immune responses, we reasoned that a therapeutic vaccine against JCV along with IL-7 treatment for general immune restoration might be effective by preferentially expanding JCV-specific CD4<sup>+</sup> T cells over other specificities. Here, we have treated two PML patients, who suffered from hereditary or acquired immunocompromise, with three subcutaneous injections of recombinant human IL-7 (r-hIL-7, CYT107) and a therapeutic vaccine consisting of JCV VP1 protein in combination with a topically administered toll-like receptor 7 (TLR7) agonist as adjuvant [8].

## **METHODS**

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### **Ethics Statement**

Both patients received the above treatment as "Compassionate Use". The term "Compassionate Use" refers to use of a non-approved drug or biological/chemical compound outside the framework of a clinical trial. The physician acts here in the scope of her/his medical license and takes complete responsibility for the prescribed therapy. When using a non-approved medication, the patient must be adequately informed and special authorization at the local Agency for Therapeutic Products obtained. Both patients were treated in full compliance with regulatory requirements in Germany and Switzerland. Patients and relatives were informed of the risks of the treatment including death from PML-IRIS and signed an informed consent.

### **Patients**

Clinical information, neuroimaging- (MRI), virological- and immunological findings are summarized in Supplementary Table I. Both patients significantly deteriorated during the 12 months between diagnosis and treatment, and cerebrospinal fluid (CSF) JCV DNA remained positive during this time. Both patients were HIV negative.

### **Treatment Regimen and Follow-up**

Patients have been treated with three subcutaneous (s.c.) injections of glycosylated recombinant human IL-7 (r-hIL-7; 10µg/kg body weight, CYT107, Cytheris S.A., Paris, France), three s.c. injections of 1 mg of recombinant JCV VP1 protein (Life Science Inkubator, Bonn, Germany) and topical imiquimod cream at the s.c. injection site (5%, Aldara®, MEDA Pharm, Germany), a TLR7/8 agonist used as adjuvant [8]. Treatment protocol is summarized in Figure (upper scheme).

Tolerability and safety were assessed by clinical and laboratory adverse events (AEs, Institute of Clinical Chemistry, University Medical Center Hamburg Eppendorf, and the Institute of Clinical Chemistry, Hematology- and Neurology Clinics, University Hospital Zürich). Efficacy assessment included JCV DNA load, MRI, clinical observation and immunological testing. Cranial MRI examinations (T1 pre- and post gadolinium-; proton density (PD)/T2-; fluid attenuated inversion recovery (FLAIR)-, and diffusion-weighted images) were performed at the Department of Neuroradiology, University Medical Center Hamburg-Eppendorf, and the Institute for

Neuroradiology, University Hospital Zurich. Scripps neurological rating scale (SNRS) score was used to assess the clinical status (SNRS, 100 normal - 0 dead).

### **Quantification of JCV Viral Load**

Viral load was quantified in the CSF by quantitative PCR (qPCR) of JCV T antigen as previously described [9].

### **Proliferative Assays**

Proliferative response of peripheral blood mononuclear cell (PBMCs) against VP1 (2 µg/mL, [10]) and tetanus toxoid (TTx, 10 µg/mL, Novartis Behring, Marburg, Germany,) was tested in a 7-day 3H-thymidine incorporation assay, and stimulatory indices (SI) calculated as: mean counts per minute (cpm) with protein / mean cpm unstimulated wells. Proliferation was also determined by flow cytometry using CellTrace™ CFSE kit (Invitrogen, Darmstadt, Germany) in PBMCs seeded with VP1 protein (2 µg/mL) for six days, labeled with CFSE and restimulated or not with VP1. After five days cells were stained with anti-CD4 (Biolegend, San Diego, CA), anti-CD3 (eBioscience, San Diego, CA), anti-CD25 (Biolegend), and anti CD45RO (Biolegend). Samples were acquired using LSR-II and LSR-Fortessa flow cytometers (BD, Franklin Lakes, NJ) and data analyzed using FACS Diva Software (BD).

### **ELISA for VP1-Specific Antibodies**

VP1-specific IgG titers in CSF/serum were determined as previously described [11]. Samples were pre-adsorbed with soluble BKV VP1 to compete potentially crossreactive antibodies. Virus-specific antibody indices (AI) were calculated as previously described [12]. Values of AI ≥ 1.5 indicate intrathecal antigen-specific antibody synthesis.

### **Statistical Analysis**

Statistical analyses were performed with Prism 5.02 (GraphPad Software Inc., San Diego, CA). Descriptive statistics are reported as mean ± SEM. Comparisons of three groups and more were assessed by one-way ANOVA with Bonferroni's correction for multiple comparisons. P-values < 0.05 were considered statistically significant.

## RESULTS

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### Safety and Tolerability

Only patient 1 reported a mild AE (swelling and reddening at the r-hIL-7 injection site). No hematological or blood chemistry abnormalities were observed, and all compounds were tolerated well.

### Efficacy

A clear reduction in the CSF JCV load was observed in both patients (Figure-A). JCV viral load testing remained negative during follow-up (12 months). Regarding MRI, no new T2 lesions were observed after treatment (Figure-B). Contrast-enhancing lesions never occurred in either patient before treatment over a 12 month period. After treatment we observed a subtle Gd-enhancing MRI lesion in patient 1, and clear enhancement in patient 2 indicative of an immune response in PML lesions (Figure-B). Clinically stabilization and/or amelioration of neurological findings after treatment was observed in both patients (see Scripps Neurological Rating Scale, SNRS, scores in Figure-C). Patient 1 significantly deteriorated during the twelve months between diagnosis and treatment (SNRS score dropped from 78 to 71). At the time of treatment he showed bilateral cerebellar signs of the lower limbs with gait ataxia and severe aphasia with leading comprehension deficits. Following treatment, he stabilized and perceptibly improved regarding cerebellar signs, speech and cognitive functions while remaining stable during follow-up (SNRS score 80). Patient 2, who had steadily deteriorated before treatment developing left-sided hemiplegia and largely bedridden (SNRS score 49), stabilized clinically after treatment with signs of mild neuropsychological improvement regarding alertness (SNRS score 53). Finally, JCV VP1-specific CD4<sup>+</sup> T cell responses served as mechanistic efficacy measure. Before treatment, PBMCs of both patients failed to respond to VP1 protein despite JCV infection. After treatment, proliferation of VP1-specific CD4<sup>+</sup> T cells rose significantly in both patients (Figure-D). CFSE-labeled PBMCs from patient 1 after treatment and stimulated in vitro with VP1 confirmed that proliferating cells were mainly memory CD4<sup>+</sup> T cells, although CD8<sup>+</sup> T cell proliferation also rose (Supplementary Figure 1A). T cell responses to the recall antigen TTx were normal before treatment in patient 1 and remained unchanged at the end of treatment. In patient 2, TTx-specific CD4<sup>+</sup> T cell responses were negative but turned positive after treatment most likely as an effect of immune reconstitution by r-hIL-7 (Figure-D). Supporting this notion the abnormally low CD4/CD8 ratio in both patients increased to normal levels after treatment (Supplementary Figure 1B). Intrathecal VP1-specific

antibody responses were elevated before treatment in both patients and did not change after treatment (Supplementary Figure 1C).

## **DISCUSSION**

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PML is often fatal in immunocompromised patients, and there is currently no treatment. Only immune reconstitution with r-hIL-7 (CYT107) represents an option based on preliminary data. Here, we performed two individual treatment attempts following ethical guidelines in two PML patients, who suffered from hereditary or acquired CD4 lymphopenia. Although these two PML cases could be seen as not sufficiently representative since they showed a longer survival than the majority of untreated PML patients, they significantly deteriorated during the twelve months between diagnosis and treatment. Due to the often fatal outcome of PML patients, they were treated with subcutaneous injections of r-hIL-7 (CYT107) with the aim to restore overall immune function. We have previously shown that JCV VP1-specific CD4<sup>+</sup> T cells are highly enriched in the brain during PML-immune reconstitution inflammatory syndrome (IRIS) and probably critical for eliminating JCV from the CNS [3]. Since restoration of immune competence may not be sufficient to expand these JCV VP1-specific CD4<sup>+</sup> T cells even in the presence of JCV and VP1 in the brain, we decided to also vaccinate patients with JCV VP1 protein via an immunogenic route, e.g. subcutaneously, in combination with a toll-like receptor 7 agonist as adjuvant in order to assure boosting of VP1-specific CD4<sup>+</sup> T cell responses over other specificities. This PML treatment was not only well tolerated, but most importantly also appears to have long-lasting efficacy with respect to elimination of CSF JCV viral load, induction of MRI-proven inflammation at sites of PML lesions without prominent/classical PML-IRIS and clinical stabilization with slight improvement. This efficacy means that the two aims of our treatment, to restore overall immune responsiveness and to boost JCV VP1-specific CD4<sup>+</sup> T cells, were accomplished. Immune restoration by r-hIL-7 was evident in patient 2, who had a low response to the recall antigen TTx and who regained responsiveness after treatment. Along this line, the peripheral CD4/CD8 ratio was also normalized after treatment in both patients. Further, CD4<sup>+</sup> JCV VP1-specific T cell responses increased significantly from previously negative in both patients. Despite these encouraging results, additional investigation of treatment efficacy in PML patients with more severe



immunocompromise is warranted to discern the relative contribution of each compound and the putative role of VP1-specific CD8<sup>+</sup> T cells. Furthermore, although this therapeutic approach is not suitable for HIV or monoclonal antibody treatment-associated PML patients, in whom immune reconstitution is not the limiting factor, we believe that HIV patients or patients who will start on or are already treated with therapies that pose the risk of PML might benefit from a prophylactic vaccination with VP1.

## **FUNDING**

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The Institute for Neuroimmunology and Clinical Multiple Sclerosis Research was supported by the Gemeinnützige Hertie Stiftung. The Section of Neuroimmunology and Multiple Sclerosis Research (nims) is supported by the Clinical Research Priority Program MS (CRPP<sup>MS</sup>) of the University Zurich.

## **CONFLICT OF INTEREST**

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Therese Crouchs is an employee of Cytheris, which produces r-hIL7 (CYT107). M. Sospedra, S. Yousef, I. Jelcic, S. Schippling and R. Martin are co-inventors of a patent application held by the University of Zurich. The other authors do not have conflicts of interest to disclose.

## **ACKNOWLEDGEMENT**

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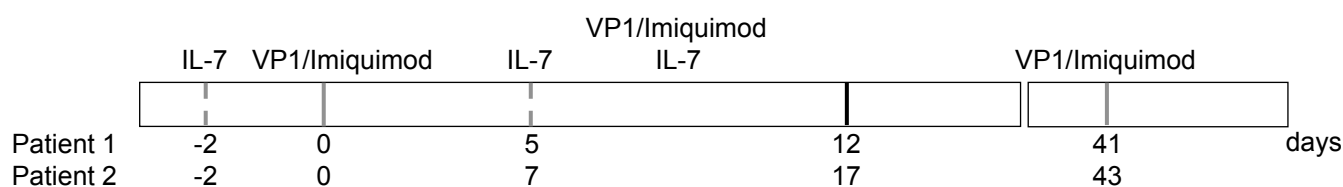
We thank Björn Zörner and Nikolai Pfender for assistance in clinical management, Brenda Reinhart for carefully reading the manuscript and Magdalena Foege for preparation of regulatory documents, all at Neuroimmunology and MS Research, Department of Neurology, University Zurich.

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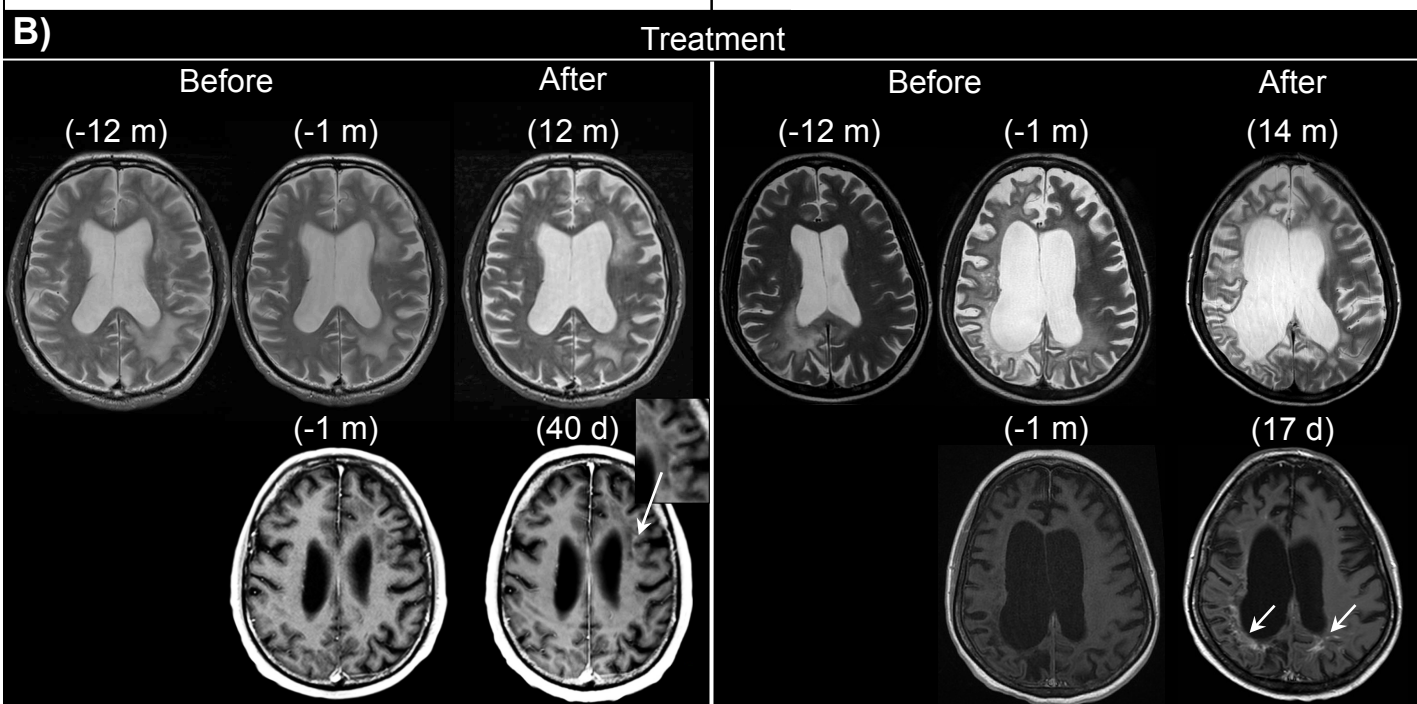
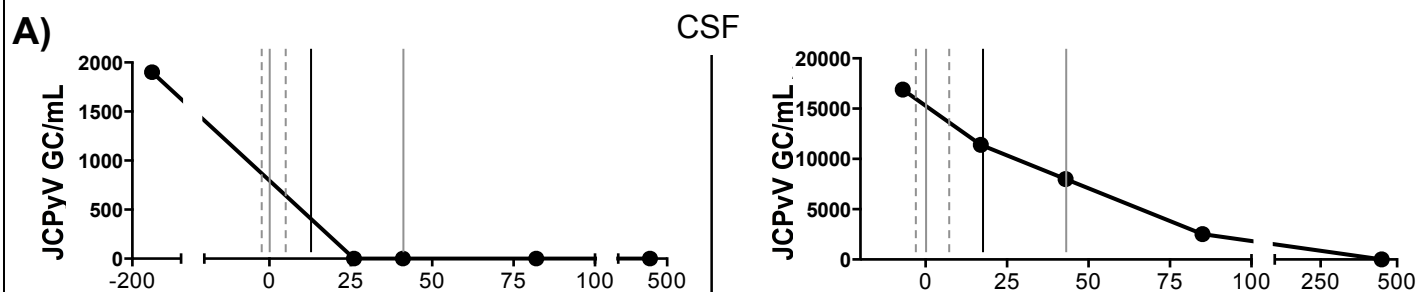
## FIGURE LEGEND

Treatment Protocol (upper scheme). Dotted grey lines: time points of r-hIL-7 injection (patient 1: days -2 and 5; patient 2: days -2 and 7), solid grey lines represent time points of VP1 injection in combination with imiquimod (patient 1: days 0 and 41; patient 2: days 0 and 43), and black solid line represents simultaneous injection of VP1 and r-hIL-7 in combination with imiquimod (patient 1: day 12; patient 2: day 17). Day 0 is the day of the first VP1 injection. Subtle differences in schedule between the two patients were due to an intercurrent urinary tract infection in patient 2. **A)** JCV viral load in CSF from patient 1 (lower left graph) and patient 2 (lower right graph) before and at different time points during and after treatment. Graphs: y-axis represents viral load expressed as viral genome copies (GC)/mL and x-axis time in days. **B)** T2 MRI images from patient 1 (upper left) and patient 2 (upper right) before treatment and 12/14 months after treatment. Contrast-enhanced T1W MRI in patient 1 (low left) and in patient 2 (low right) performed before and 40 days (patient 1) or 17 days (patient 2) after first VP1 injection. White arrows point at Gd contrast-enhancement indicative of neuroinflammation in the areas of PML lesions in both patients. The inset in the image of patient 1 focuses on the band-like Gd contrast-enhancing lesion. (m=months, d=days) **C)** SNRS before treatment and 3 months and 12/14 months after treatment. \* Not available. **D)** JCV VP1- and TTx-specific CD4<sup>+</sup> T cell responses before (month -1, white histograms), during (patient 1: day 12 and 41, patient 2: day 17 and 43; grey histograms) and after (day 84, black histograms) r-hIL-7/JCV VP1 vaccination treatment. Proliferative responses were measured by 3H-thymidine incorporation assay. Graphs (y-axis) represent SI (stimulation index). Mean  $\pm$  SEM and statistical significance are shown, \* $p<0.05$ , \*\* $p<0.01$  and \*\*\* $p<0.001$ .



**Patient 1**

**Patient 2**



**C)**

	Treatment			
	Before	Before	After	After
	-12	-1	3	12
SNRS	78	71	80	80

	Treatment			
	Before	Before	After	After
	-12	-1	3	14
months	NA*	49	53	53

